



TCBS Agar

Selective medium for detection of enteropathogenic *Vibrio* spp from clinical and nonclinical samples, according to ISO 21872.

DESCRIPTION

Thiosulfate Citrate Bile Sucrose (TCBS) Agar is a medium used for the selective isolation and cultivation of vibrios.

This medium conforms to ISO 21872 for the identification of *Vibrio* spp, including *Vibrio cholerae* from food, animal feeding stuffs and environmental samples in the area of food production and food handling.

TCBS Agar is also recommended for isolating *V. cholerae* and *V. parahaemolyticus* as well as other vibrios from stool specimens.

TYPICAL FORMULA

	(g/l)
Peptone	10.0
Yeast Extract	5.0
Sodium Citrate	10.0
Sodium Thiosulfate	10.0
Iron(III) Citrate	1.0
Sodium Chloride	10.0
Dried Bovine Bile	8.0
Sucrose	20.0
Bromothymol Blue	0.04
Thymol Blue	0.04
Agar	15.0
Final pH 8.6 ± 0.2 at 25°C	

METHOD PRINCIPLE

Peptone and yeast extract provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Sodium citrate serves to maintain an alkaline pH and along with sodium thiosulfate and oxbile are selective agents, inhibiting Gram-positive organisms and suppressing coliforms. Moreover, the alkaline pH of the medium enhances the recovery of *V. cholerae*. Sodium thiosulfate serves also as a sulfur source and, in combination with ferric citrate, detects hydrogen sulfide production. Sodium chloride maintains the osmotic balance of the medium and stimulates vibrios growth. Sucrose is the fermentable carbohydrate. Bromothymol blue and thymol blue are pH indicators. Agar is the solidifying agent.

PREPARATION

<u>Dehydrated medium</u>	Suspend 89.1 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. DO NOT AUTOCLAVE.
<u>Medium in bottles</u>	Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into final containers.

TEST PROCEDURE

ISO 21872 recommends to follow two successive enrichment steps in Alkaline Saline Peptone Water (ASPW) before inoculating TCBS Agar.

TCBS Agar can be also inoculated directly with specimens such as rectal swabs, feces, vomitus or with food samples (*).

Incubate (protected from light) at 37 ± 1°C for 18-24 hours in an aerobic atmosphere.

(*) NB. Heavy inoculation is recommended. Swabs containing specimen material should be transported to the laboratory in Cary Blair Transport Medium (ref. 470290) if a delay in reaching the laboratory is anticipated. Specimens for cultivation of vibrios should not be frozen.

INTERPRETING RESULTS

Strains of *Vibrio cholerae* produce yellow colonies on TCBS Agar because of fermentation of sucrose. *Vibrio alginolyticus* also produce yellow colonies. It's possible that a few sucrose-positive *Proteus* strains can grow to form yellow, vibrid-like colonies. *Vibrio parahaemolyticus* is a sucrose non-fermenting organism and produces blue-green colonies, as does *Vibrio vulnificus*. Occasional isolates of *Pseudomonas* and *Aeromonas* species also produce blue-green colonies, but overall TCBS Agar is highly selective and any H₂S-negative colony is possibly *Vibrio* species.

APPEARANCE

Dehydrated medium: free-flowing, homogeneous, light beige to green beige.

Prepared medium: clear to slightly opalescent, green.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles, tubes and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
 Medium in bottles: 2 years.
 Medium in tubes: 1 year.
 Ready-to-use plates: 6 months.

QUALITY CONTROL

The medium is inoculated with the microbial strains indicated in the QC table.
 Inoculum for productivity: ≤ 100 CFU.
 Inoculum for selectivity: $> 10^3$ CFU.
 Incubation conditions: aerobically at $37 \pm 1^\circ\text{C}$ for 18-24 hours.

QC Table.

Microorganism		Growth	Colony Color
<i>Vibrio parahaemolyticus</i>	WDCM 00185	Good	Green
<i>Vibrio furnissii</i>	WDCM 00186	Good	Yellow
<i>Escherichia coli</i>	WDCM 00012	Inhibited	---

WARNING AND PRECAUTIONS

The product does not contain hazardous substances in concentrations exceeding the limits set by current legislation and therefore is not classified as dangerous. It is nevertheless recommended to consult the safety data sheet for its correct use. The product is intended for *in vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL OF WASTE








Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

- EN ISO 11133:2014. Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.
- ISO 21872-1:2007. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. – Part 1: Detection of *Vibrio parahaemolyticus* and *Vibrio cholerae*.
- ISO 21872-2:2007. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. – Part 2: Detection of species other than *Vibrio parahaemolyticus* and *Vibrio cholerae*.
- American Public Health Association (1992): compendium of methods for the microbiological examination of foods, 3rd edition.
- Dewitt, W.E., E.J. Gangarosa, I. Huq, and A. Zarifi (1971) Holding media for the transport of *Vibrio cholerae* from field to laboratory. Am. J. Trop. Med. Hyg. 20:685-688.
- Kobayashi, T., S. Enomoto, R. Sakazaki, and S. Kuwahara (1963) A new selective medium for pathogenic vibrios: T.C.B.S. Agar (Modified Nakanishi's Agar). Jap. J. Bacteriol. 18:387-391.

PRESENTATION		Contents	Ref.
TCBS Agar	90 mm ready-to-use plates	20 plates	11195
TCBS Agar	90 mm ready-to-use plates	100 plates	11195*
TCBS Agar	Slant tubes	10 x 9 ml tubes	30022
TCBS Agar	Slant tubes	20 x 9 ml tubes	31022
TCBS Agar	Bottles	6 x 100 ml bottles	403140
TCBS Agar	Dehydrated medium	500 g of powder	611010
TCBS Agar	Dehydrated medium	100 g of powder	621010

TABLE OF SYMBOLS

LOT Batch code	IVD <i>In vitro</i> Diagnostic Medical Device	 Manufacturer	 Use by	 Fragile, handle with care
REF Catalogue number	 Temperature limitation	 Contains sufficient for <n> tests	 Caution, consult Instruction For Use	 Do not reuse



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